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Phenotypic plasticity and developmental innovations in nematodes

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Developmental plasticity has been implicated as a facilitator for phenotypic diversification, but the molecular mechanisms controlling it are largely unknown. We review recent comparative analyses in non-Caenorhabditis nematodes that display polyphenisms in larval development, mouth morphology and reproductive mode. Some of the challenges ahead will be to connect how these phenotypic traits are linked to each other at the molecular level, and at the ecological level. This will require sampling of several nematode species, the characterization of their ecology and the employment of both classical genetics and recently developed technological advances, such as genome editing.

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Introduction

Natural selection shapes the evolution of populations, communities and ecosystems by acting on phenotypes. It is through the elimination of poorly adapted traits that populations change, diversify, specialize or become extinct. Phenotypic plasticity — i.e., the ability of an organism to produce different phenotypes in response to its environment — offers the possibility for organisms to adapt to varying environments in real time. Plasticity can thus be studied as a trait under selection: organisms unable to respond adaptively to environmental pressures by lack of plasticity or maladaptive plasticity will be eliminated whereas those exhibiting adaptive plasticity will be selected for. How phenotypic plasticity evolves and how it, in turn, influences evolution, are fundamental questions in modern biology.

It has been argued that increased plasticity enables the appearance of new phenotypes and thus promotes diversification, population divergence and speciation [1–4]. Conversely, it has also been put forward that plasticity can prevent diversification. Indeed, if one plastic genotype can result in various phenotypes, the emergence of alternative genotypes becomes unnecessary. Additionally plasticity can hide genetic diversity since one (optimal) phenotype can originate from multiple genotypes (canalization) [2,4].

Despite the ecological and evolutionary importance of plasticity, there is little experimental evidence that explains the underlying molecular and physiological mechanisms controlling such traits or how they evolved. Moreover, the interplay between phenotypic plasticity, ecology and evolution is further complicated by the fact that the plasticity of one trait can influence the phenotype of another, later in the developmental trajectory or in the life cycle of a species [5••,6••]. How plastic traits become linked, and the ecological and evolutionary consequences of such connections remain unclear.

Nematodes are a model of choice to study the mechanisms and evolution of phenotypic plasticity [7]. This widespread species-rich group displays a tremendous array of life-styles and adaptations, which make them ideal for evolutionary, developmental and ecological studies. Moreover, the simplicity of their morphology makes them an easy model to tackle these Eco-Evo-Devo questions at the molecular (genetic) and organismal (physiological) levels.

In this mini-review, we relate recent findings on the development of phenotypic plasticity in nematodes, particularly on the link between dauer polyphenism and ecologically relevant traits such as reproduction and diet. The focus is placed on non-Caenorhabditis nematodes, and especially *Pristionchus*, a model particularly attractive for evo-devo studies, since it is amenable to classical and modern genetics techniques (genome editing with CRISPR/Cas9) [8].

Dauer formation and adult mouth morphology

Pristionchus species have two types of polyphenism, both of which are influenced by environmental cues experienced during larval development. The first type of polyphenism, which can also be found in other nematodes including *Caenorhabditis elegans*, is the facultative formation of a non-feeding larval stage. The second type of

polyphenism, which is specific to the diplogasterid clade of nematodes that includes *Pristionchus*, is reflected in the mouth morphology of the last larval stage and the adult. To understand the evolution and adaptive significance of these polyphenisms, and the link between them, it is useful to first consider *Pristionchus* in its ecological context.

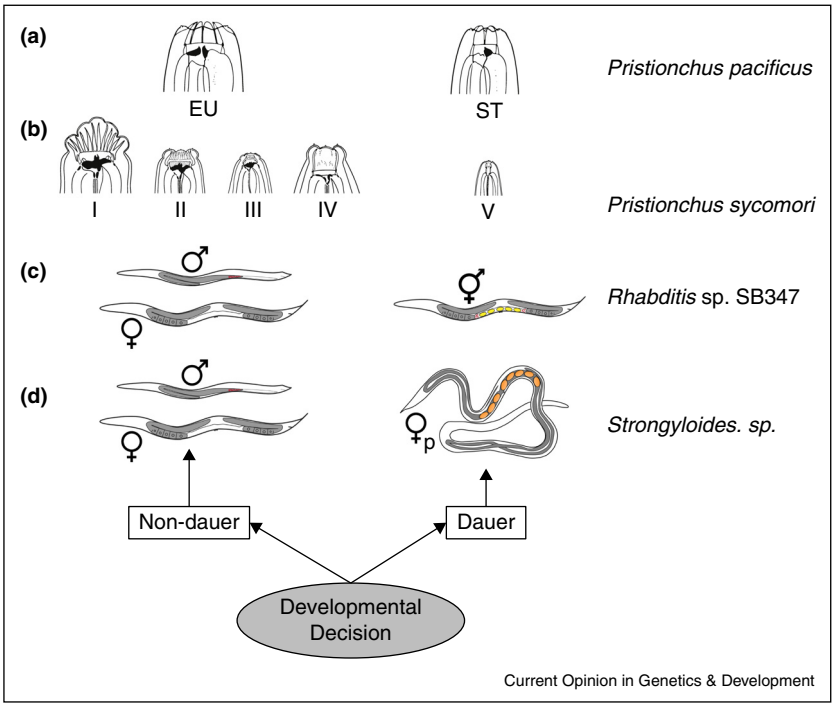
The best-known *Pristionchus* species, *P. pacificus*, is a free-living nematode that associates with scarab beetles. The beetles are used as vectors for dispersal and substrate for the growth of microbial food [9–12]. When on live beetles, *P. pacificus* is typically found in an arrested larval stage named 'dauer', and can remain in this non-feeding stage for up to a year [13]. After the death of the beetle, microbial growth on the carcass triggers *P. pacificus* to resume its larval development to become a reproductive adult [14,15].

Self-fertilizing hermaphrodite adults typically produce over 100 progeny for 4–5 days [13,16], resulting in high population densities in a relatively short period of time. This fast population growth becomes challenging for their own survival, because of competition with other individuals for rapidly depleting food resources. Pheromones present in dense nematode populations guide larvae towards developmental choices that equip *P. pacificus* to cope with these stressful conditions. Larvae may develop either into adults that can efficiently eat additional diets besides bacteria, or arrest development as the starvation-resistant dauers [17]. This developmental choice is highly dependent on the type of pheromone to which the juveniles are exposed [18], maternal phenotype [19•], and the nematode genetic background [20,21].

Larvae exposed to specific pheromones (e.g., part#9, ubas#1 and npar#1) develop into dauers [18,21], which have biochemical and behavioral traits suited for dispersal [22]. After exiting dauer, the resulting *P. pacificus* adults mostly develop a narrow mouth [6••], referred to as stenostomatous (ST) [23,24] (Figure 1a). Larvae that bypass dauer can develop into adults with either a ST mouth or a broader mouth named eurystomatous (EU) [23]. Those EU nematodes have two large teeth that move as scissors to break open fungal spores and nematodes [23,24]. This mouth morph develops more often when the larvae are starved or when exposed to pheromones (e.g., dasc#1, pasc#9 and npar#1) [18]. Thus, *P. pacificus* can become carnivorous and survive on a non-bacterial diet [25].

The link between dauer formation and mouth polyphenism has been recently extended to other *Pristionchus*

Figure 1



Simplified diagram of links between polymorphisms in nematodes. Coupling of polymorphisms can occur between dauer decision and mouth form **(a, b)**, and between dauer decision and sexual morph **(c, d)**. For *Strongyloides* sp. **(d)**, females can be obligate outcrossers (♀) or parthenogenetic (♀p). Cross-generational factors, environmental factors and the individual's genetic background can influence the developmental decisions.

species, which may have more than two mouth morphs [26••]. The fig and wasp-associated *P. sycomori*, for instance, displays five mouth morphs (Figure 1b). Interestingly, all animals that pass through dauer develop a specific mouth morph (morph V). The remaining four morphs are produced in subsequent generations, from animals that do not pass through dauer. The mouth morphology of morph V of *P. sycomori* is similar to the ST mouth of *P. pacificus*, and both of these are mostly microbivorous.

Dauer formation and mode of reproduction

Another example in which two types of polyphenisms are linked is found in the free-living nematode provisionally named *Rhabditis* sp. SB347. *Rhabditis* sp. SB347 XX larvae that go through dauer become hermaphrodites, and larvae that bypass dauer become females (XO larvae never become dauers and develop into male adults) [5••,27] (Figure 1c).

Some nematode parasites also couple a type of larval stage with the mode of reproduction [28–30]. The free-living generation of *Strongyloides*, composed of males and females, mate to produce larvae that are specialized in infecting a mammalian host. Those larvae invariably become parthenogenetic adults that live their entire adult lives within the host mammal. The progeny of those females, in turn, generate free-living larvae that develop into obligatory outcrossers males and females [30]. Thus, *Strongyloides* adults are able to reproduce by parthenogenesis or outcrossing, depending on whether they passed through an infective stage or not. Interestingly, infective larvae of parasitic nematodes like *Strongyloides* share many similarities with the dauer larvae of free-living nematodes (Figure 1d), including a thin body, thickened cuticle, a non-feeding closed mouth, and their development is controlled by the same transcription factor [31,32].

The role of DA/DAF-12 in the link between polyphenisms

Relatively little is known about how dauer formation is linked to other types of polyphenism. The nuclear hormone receptor DAF-12, a homolog of vertebrate vitamin D and liver X receptors [31], is likely to be a key player in the evolution of polyphenism because it affects not only dauer formation but also other types of polyphenism. Best studied in *C. elegans*, biochemical analysis suggests that cholesterol-derived ligands named dafachronic acid (DAs) regulate DAF-12 activity [33,34]. The synthesis of DAs is regulated by the integration of environmental signals and a complex neuroendocrine signaling network [35,36].

Exposure of *P. pacificus* to exogenous DAs, or mutations in *daf-12*, prevents dauer formation [17] and results in ST adults [6••]. These studies suggest that, in addition to its role in regulating dauer formation, DAF-12 evolved new target genes in *P. pacificus* that directly or indirectly regulate mouth polyphenism. A possible target for DAF-12 is *eud-1*, a gene coding for a sulphatase that when mutated results in ST worms [37•]. *P. pacificus* mutants for the gene *daf-16*, which codes for another transcription factor necessary for dauer formation, display mouth polyphenism [38•]. Thus, this indicates that dauer formation is sufficient to induce a high percentage of ST morphs, but it is not necessary for the development of this mouth morph. Indeed, the ST mouth can develop from individuals that have not passed through dauer [6••].

The dauer-mouth morphology link in *P. pacificus* contrasts with the dauer-sex link found in *Rhabditis* sp. SB347. In the former, dauer formation does not influence the mouth morphology in every case [6••], but in *Rhabditis* sp. SB347 it always influences sex determination [5••]. In *Rhabditis* sp. SB347, dauer formation is necessary *and* sufficient to determine the hermaphrodite sex [5••]. Indeed, by artificially repressing dauer with DAs, or inducing dauer by removing/inhibiting the precursor of DAs, it is possible to induce the development of the female or the hermaphrodite sexual morphs, respectively. The triggering factor determining the developmental pathway/sexual morph has yet to be found. As SB347 dauer formation (and sexual fate) can be manipulated with DAs indicates that DAF-12 is probably also involved in the link between dauer and reproductive mode.

In a similar fashion, the infective stage and adult mode of reproduction of *Strongyloides* can be changed by the treatment of juveniles with DAs [17,32]. The DA-treated larvae bypass the infective stage and develop into female adults, which can only reproduce by outcrossing. The fact that it is possible to prevent the infective stage by applying exogenous DA, and that DAs can bind to DAF-12 in *Strongyloides* suggests that the DA-DAF-12 module has been co-opted in this lineage. Under this hypothesis, the ancestral role of this module is to regulate dauer formation, and in *Strongyloides* the DA-DAF-12 module plays the additional role of regulating sex determination, which correlates with infective or free-living individuals.

However, further work is necessary to test this hypothesis of co-option, such as the generation of mutants for *daf-12* in *Rhabditis* sp. SB347 and other species displaying the dauer/sex link. The identification of regulators and targets of the DA biosynthesis pathway and of DAF-12 will also be important to unravel the molecular players responsible for both the dauer-sex and dauer-mouth links. For instance, although *P. pacificus* DAF-12 can respond to *C. elegans* DAs, those molecules do not seem to be present in the *P. pacificus* metabolome (FC Schroeder, unpublished data). To understand how the activity of DAF-12 is controlled in *P. pacificus*, the regulation of the synthesis of the ligands of this protein also have to be uncovered.

Maternal control of polyphenisms and ecology

Interestingly, maternal effects can affect the mouth morphology of *P. pacificus*. In this species, the maternal phenotype biases the phenotype of the male progeny. Whereas ST mothers generate no ST males, EU mothers produced about 30% of ST males [19•]. The cross-generational signal that biases the formation of ST males from EU mothers has yet to be identified, but the generation of sex determination mutants may help to answer this question [39]. The ecological significance of ST and EU, and their link to maternal phenotype and to dauer, is still not clear, although ST worms were found to develop a few hours faster than EU worms [19•]. In *P. sycomori*, maternal effects could also be playing a role, since the maternal phenotype seems to determine the range of mouth polymorphisms. For example, dauer/mouth type V individuals do not produce progeny that display the maternal phenotype, but rather progeny that display mouth types I–IV [26••]. The five morphs vary in mouth width, size and number of the teeth, and labial morphology, reflecting their different types of diet.

In most free-living nematodes, the first larval stages respond to the environment by either arresting as dauer or continuing development towards adulthood. In *Rhabditis* sp. SB347, however, more than half of the worms arrest as dauer larvae, independently of the environmental conditions they experience [5••,27]. Thus, in contrast to *C. elegans* and *P. pacificus*, dauer entry in *Rhabditis* sp. SB347 is not dependent on environment cues experienced by the larvae. Instead, the larvae are pre-specified by the mother to become dauer. The likelihood of becoming dauer correlates with the age and sex of the parent: hermaphrodite mothers produce more female offspring than female mothers and, additionally, the older the parent, the higher is the likelihood for the animal to develop through the dauer stage and become a hermaphrodite [5••,40]. Further research is required to elucidate how this occurs. One possibility is that the mother relays a dauer-inhibiting factor to the early progeny.

The constant production of dauers by *Rhabditis* sp. SB347 is likely to be a bet-hedging strategy to withstand rapid environmental changes [41]. Thus, dauers that disperse to favorable environments develop into hermaphrodites, which can reproduce without a mating partner. Interestingly, in an undescribed species that is closely related to *Rhabditis* sp. SB347 (provisionally named *Rhabditis* sp. SB372), it is the mother that responds to the environment, not the larvae. In this species, unfavorable environmental conditions induce the mother to shift from the production of non-dauer larvae that develop into males and females to the production of dauers, which later develop into hermaphrodites (pers. obs.). The regulation of the phenotype of the progeny by environmental conditions experienced by the mother is reminiscent of diapause regulation found in some insects [42]. Future research will reveal the nature of the maternal signal that determines dauer entry in the progeny in *Rhabditis* sp. SB347 and other nematodes.

Evolutionary history of developmental innovations

The mechanisms leading to the coupling of the dauer pathway to mouth polyphenism in *Pristionchus* or sexual morph in SB347 are intriguing, but it remains to be seen if these links are adaptive and actively selected for, or if they are merely a consequence of shared underlying developmental processes. The uncoupling of the traits and/or the comparison between strains and species differing in the degree of coupling will help us understand how these links occurred and evolved as well as their ecological significance.

Beyond the linkage, the polyphenisms of mouth forms in *Pristionchus* and sexual morph of SB347 are both of great ecological (and evolutionary) significance. They provide a context of choice to investigate the origin and evolution of alternative lifestyles. The appearance of a new mating system (through the emergence of a new sex) has profound effects on the genetics and evolution of populations and it is possible that it led to the diversification of lifestyles, such as parasitism. The mouth form innovations observed in *Pristionchus* species render accessible new ecological niches permitting a diversification of lifestyle intra-species [26••] and throughout the evolution of the group [3]. Moreover, these polyphenisms offer the possibility to study how evolutionary forces act on plastic traits and how in turn plasticity can affect evolution. With the exciting technological advances such as genome editing, new discoveries are opening up for satellite models such as *Pristionchus* and *Rhabditis* sp. SB347.

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